

What is claimed:

1. An isolated peptide inhibitory to bacterial growth
selected from the group consisting of enterocin 900
(SEQ ID NO:28), brochocin-C (SEQ ID NO:23), and
muteins thereof.
2. The peptide of claim 1, wherein said peptide is
enterocin 900 or a mutein thereof.
3. The peptide of claim 1, wherein said peptide is
brochocin-C peptide A, brochocin-C peptide B, or a
mutein thereof.
4. An isolated polynucleotide, said polynucleotide
comprising a structural gene sequence which encodes
a peptide inhibitory to bacterial growth selected
from the group consisting of enterocin 900 (SEQ ID
NO:28), brochocin-C (SEQ ID NO:23), and muteins
thereof.
5. The polynucleotide of claim 4, further comprising a
promoter operable in a host microorganism, operably
linked to said structural gene sequence.
6. The polynucleotide of claim 5, wherein said
microorganism comprises a lactic acid bacterium.
7. The polynucleotide of claim 6, further comprising a
bacteriocin processing peptide operable in said

host microorganism, operably linked to said structural gene sequence.

5 8. The polynucleotide of claim.10, wherein said bacteriocin processing peptide comprises a divergicin A processing peptide.

10 9. A secretion vector, for providing secretion of a heterologous protein from a microbial host cell, said secretion vector comprising:

a first polynucleotide encoding a heterologous protein;

a second polynucleotide encoding a bacteriocin processing peptide operable in said host cell, operably linked to said polynucleotide encoding said heterologous protein; and

15 a promoter operable in said host cell, operably linked to said polynucleotide encoding said heterologous protein.

20 10. The secretion vector of claim 9, wherein said bacteriocin processing peptide comprises a divergicin A processing peptide or an operable mutein thereof.

25 11. The secretion vector of claim 9, wherein said first polynucleotide encodes a heterologous bacteriocin.

30 12. The secretion vector of claim 11, further comprising an immunity gene which confers immunity from said heterologous bacteriocin to said host cell.

13. The secretion vector of claim 11, wherein said vector encodes a plurality of different bacteriocins.

5 14. The vector pCD3.4 (SEQ ID NO:14).

15. A host cell transformed with the secretion vector of claim 12.

10 16. A host cell transformed with a plurality of secretion vectors of claim 12.

17. A host cell transformed with the secretion vector of claim 13.

15 18. A method for inhibiting the growth of susceptible bacteria in an environment, comprising:

providing a microorganism capable of expressing a bacteriocin selected from the group consisting of enterocin 900, brochocin-C, and operable muteins thereof; and

20 applying said microorganism to said environment in an amount sufficient to inhibit the growth of susceptible bacteria.

25 19. The method of claim 18, wherein said environment comprises a foodstuff.

20. The method of claim 19, wherein said foodstuff comprises meat.

30 21. The method of claim 18, wherein said environment

comprises a living mammal.

22. The method of claim 21, wherein said environment
comprises a food preparation area.

23. The method of claim 21, wherein said microorganism
expresses a plurality of bacteriocins.

24. The method of claim 21, wherein said environment
comprises a fermentation vessel.

25. A method for inhibiting the growth of susceptible
bacteria in an environment, comprising:
providing a bactericin selected from the group consisting
of enterocin 900, brochocin-C, and operable muteins thereof; and
applying said bacteriocin to said environment in an
amount sufficient to inhibit the growth of susceptible bacteria.

26. The method of claim 25, wherein said bacteriocin
inhibits bacteria which cause mastitis.

27. A method for obtaining secretion of a protein from
a microorganism, said method comprising:
providing a secretion vector comprising a promoter
operable in said microorganism, operably linked to a
polynucleotide encoding a bacteriocin processing peptide operable
in said microorganism, operably linked to a polynucleotide
encoding said protein;

transforming said microorganism with said secretion
vector; and

culturing said transformed microorganism under conditions that induce expression and secretion.

28. The method of claim 27, wherein said bacteriocin processing peptide comprises a divergicin A processing peptide.

29 ~~30~~. An expression vector for secreting a heterologous protein in a microorganism host, said vector comprising:

a polynucleotide encoding a promoter operable in said host;

a polynucleotide encoding a leucocin A processing peptide;

a polynucleotide encoding said heterologous protein;

a polynucleotide encoding lcaC (SEQ ID NO:4); and

a polynucleotide encoding lcaD (SEQ ID NO:5).

30 ~~31~~. The vector of claim 30, wherein said heterologous protein comprises leucocin A.

31 ~~32~~. An antibody specific for a bacteriocin selected from the group consisting of brochocin-C and enterocin 900.

32 ~~33~~. A method for detecting expression of a bacteriocin in a host cell, said method comprising:

providing an antibody specific for a bacteriocin selected from the group consisting of brochocin-C and enterocin 900;

contacting said host cell with said antibody; and

detecting binding or absence of binding between said

antibody and said bacteriocin.

33 34. An isolated polynucleotide, said polynucleotide comprising a sequence encoding brochocin-C immunity (SEQ ID NO:26) or an operable mutein thereof.

5 34 35. A method for purifying brochocin-C from a microorganism culture, comprising:
extracting said microorganism culture with n-butanol;
removing said n-butanol to provide an extract; and
filtering said extract by gel filtration.

10 35 36. A method for purifying enterocin 900 from a solution, comprising:
applying said solution to a hydrophobic interaction column;
15 washing said column with a solution having less than 40% ethanol;
eluting partially purified enterocin 900 from said column with a solution having about 40% or more ethanol;
applying said partially purified enterocin 900 to an ion
20 exchange column;
washing said column with a salt solution having a concentration less than about 150 mM;
eluting enterocin 900 using a salt solution having a concentration of about 200 mM.

25 36 37. The method of claim ³⁵36, further comprising:
applying enterocin 900 to a reverse-phase HPLC column;
washing said column with a solution comprising less than about 50% ethanol; and
30 eluting enterocin 900 using a solution comprising about

70% ethanol.

37 38. A method for inhibiting the growth of susceptible bacteria in an environment, comprising:

5 providing a microorganism comprising a secretion vector, said secretion vector comprising:

a first polynucleotide encoding a bacteriocin;
a second polynucleotide encoding a bacteriocin processing peptide operable in said host cell, operably linked to said polynucleotide encoding said bacteriocin; and
10 a promoter operable in said host cell, operably linked to said polynucleotide encoding said bacteriocin; and

15 applying said microorganism to said environment in an amount sufficient to inhibit the growth of susceptible bacteria.

38 39. The method of claim 38, wherein said bacteriocin processing peptide comprises a divergicin A processing peptide or an operable mutein thereof.

20 39 40. The method of claim 39, wherein said secretion vector further comprises an immunity gene which confers immunity from said heterologous bacteriocin to said host cell.

25 40 41. The method of claim ³⁹40, wherein said vector encodes a plurality of different bacteriocins.

HETEROLOGOUS TO SAID MICROORGANISM.